

Construction of Engineered Water-soluble PQQ Glucose Dehydrogenase with Improved Substrate Specificity

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This was the first study that achieved a narrowing of the substrate specificity of water soluble glucose dehydrogenase harboring pyrroloquinoline quinone as their prosthetic group, PQQGDH-B. We conducted the introduction of amino acid substitutions into the loop 6BC region of the enzyme, which made up the active site cleft without directly interacting with the substrate, and constructed a series of site directed mutants. Among these mutants, Asn452Thr showed the least narrowed substrate specificity while retaining a similar catalytic efficiency, thermal stability and EDTA tolerance as the wild-type enzyme. The relative activities of mutant enzyme with lactose were lower than that of the wild-type enzyme. The altered substrate specificity profile of the mutant enzyme was found to be mainly due to increase in K_m value for substrate than glucose. The predicted 3D structures of Asn452Thr and the wild-type enzyme indicated that the most significant impact of the amino acid substitution was observed in the interaction between the 6BC loop region with lactose.

Keywords: Pyrroloquinoline quinone (PQQ); Glucose dehydrogenase; Substrate specificity; Protein engineering; Glucose sensor; β -Propeller protein

INTRODUCTION

Glucose dehydrogenases (GDHs) harboring pyrroloquinoline quinone (PQQ) as their prosthetic group are being utilized as an enzyme sensor constituent for glucose monitoring because of their property of oxygen independence (D'Costa *et al.*, 1986; Yokoyama *et al.*, 1989; Sode *et al.*, 1993; Ye *et al.*, 1993; Katz *et al.*, 1996; Schmidt, 1997; Kost *et al.*, 1998; Yamazaki *et al.*, 2000). Two types of PQQGDHs have been reported, the membrane-bound mono-

meric PQQGDH-A and the water soluble dimeric PQQGDH-B. Despite their superior features, further improvement of PQQGDH enzymatic properties is required. This is particularly true when PQQGDH is compared with the major enzyme utilized for glucose sensing, glucose oxidase, which has better substrate specificity and operational stability. We have previously reported the improvement of co-factor binding stability, thermal stability, and substrate specificity of PQQGDH-A based on rational analyses of *Escherichia coli*-*Acinetobacter calcoaceticus* PQQGDH-A chimeric enzymes (Sode and Sano, 1994; Sode *et al.*, 1995a,b; Sode and Kojima, 1997; Yoshida and Sode, 1997; Witarto *et al.*, 1999 a,b; Yoshida *et al.*, 1999). The improvement of substrate specificity of *E. coli* PQQGDH-A was achieved by substituting His775 to Asn (Sode and Kojima, 1997).

The primary structure of PQQGDH-B is very unique and has little homology with other PQQ enzymes. We have constructed PQQGDH-B mutant libraries using error prone PCR and obtained some mutant enzymes with altered properties. Based on the sequence analyses and enzymatic characterization, we constructed a series of PQQGDH-B mutants with improved thermal stability and increased catalytic activity (Igarashi *et al.*, 1999; Sode *et al.*, 2000). However, our random mutant libraries did not yield any enzyme with narrowed substrate specificity.

Recently, the 3D structure of PQQGDH-B was reported and the fine structure of the active center was elucidated (Oubrie *et al.*, 1999a,b,c; Dewanti and Duine, 2000; Oubrie and Dijkstra, 2000). PQQGDH-B is a homo dimeric enzyme composed, with

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each subunit forming a β -propeller structure. A β -propeller protein is composed of W-motifs and loop regions that connect each strand in the W-motif. The active site as well as substrate binding site is located on one side of the β -propeller structure in a cavity composed of the loop BCs and loop DAs of 6-W-motifs. The substrate glucose is located in the cavity composed of 1D2A, 2D3A, 3BC, 4D5A and 6BC loops, and interacts with amino acid residues located within the 1D2A, 2D3A and 3BC loops (Oubrie *et al.*, 1999b). One of the characteristic properties of GDH-B substrate specificity is that PQQGDH-B also reacts with disaccharides such as lactose and maltose (Olsthorn and Duine, 1998). Loop 6BC does not have any amino acid residues which interact with glucose. However, the interaction of the loop 6BC with other loops may influence the size of the cavity.

We have therefore conducted the introduction of amino acid substitutions into the loop 6BC region with the aim of improving of the substrate specificity. We focused on the polar amino acid residues in this region, and constructed a series of site-directed mutants. Among these mutants, we found that Asn452Thr showed narrowed substrate specificity without decreasing the catalytic activity.

MATERIALS AND METHODS

Bacterial Strains and Plasmid

E. coli PP2418, in which the PQQGDH structural gene was disrupted by insertion mutagenesis (Cleton-Jansen *et al.*, 1990), was used as the host strain for the expression for each PQQGDH-B. *E. coli* BMH71-18 *mutS* and *E. coli* MV1184 were used to construct mutations by site-directed mutagenesis. All of the PQQGDH structural genes were inserted

into the multicloning site of the expression vector pTrc99A (Pharmacia, Sweden).

Genetic Manipulation

A 1.2-kbp DNA fragment containing the wild-type PQQGDH-B structural gene was obtained by PCR amplification, inserted into pTrc99A, and designated as pGB (Sode *et al.*, 2000). A *Kpn*I-*Hind*III fragment was transferred from pGB to the linearized pKF18k vector (Takara, Japan) and used for carrying out site-directed mutagenesis. The oligonucleotide primers used for this study are summarized in Fig. 1. The position of amino acid residues are defined relative to the initiator methionine residue.

Site-directed mutagenesis was performed with the Mutan-Express Km kit (Takara, Japan) according to the manufacturer's instructions. The nucleotide sequence of mutation was confirmed with an automated DNA sequencer (ABI310 Genetic analyzer) (Applied Biosystems, USA). Mutated regions were then digested with *Kpn*I and *Hind*III and the fragment was substituted into the corresponding region of pGB expression vectors.

Enzyme Preparation and Enzyme Assay

Crude enzyme preparations of wild-type and mutant PQQGDH-B samples as well as purified Asn452Thr and Asn462His samples were prepared as previously reported (Sode *et al.* 2000).

GDH activity was measured using 0.6 mM phenazine methosulfate (PMS) and 0.06 mM 2,6-dichlorophenolindophenol (DCIP) after incubation in 10 mM MOPS-NaOH (pH 7.0) containing 1 mM CaCl_2 and 1 μM PQQ for 30 min. Each activity at 100 mM glucose was calculated by monitoring the decrease in absorbance of DCIP at 600 nm.

	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466
Wild type	3'- CAT	AAT	TGA	CTA	TGA	CGG	CCT	TTA	CAG	GTT	TTT	CTA	CTA	CCG	AGT	CAT	TGT	TTA	TGT	AAT	CTT	T -5'
	Val	Leu	Thr	Asp	Thr	Ala	Gly	Asn	Val	Gln	Lys	Asp	Asp	Gly	Ser	Val	Thr	Asn	Thr	Leu	Glu	Asn
Asp448Asn	3'- CAT	AAT	TGA	TTA	TGA	CGG	CC	-5'														
				Asn																		
Asn452Thr				3'- TA	TGA	CGG	CCT	TGA	CAG	GTT	TTT	CTA	C	-5'								
								Thr														
Asn452Lys				3'- TA	TGA	CGG	CCT	TTT	CAG	GTT	TTT	CTA	C	-5'								
								Lys														
Asn452Ile				3'- TA	TGA	CGG	CCT	TAA	CAG	GTT	TTT	CTA	C	-5'								
								Ile														
Asn452His				3'- TA	TGA	CGG	CCT	GTA	CAG	GTT	TTT	CTA	C	-5'								
								His														
Asn452Asp				3'- TA	TGA	CGG	CCT	CTA	CAG	GTT	TTT	CTA	C	-5'								
								Asp														
Asp456Asn							3'- CCT	TTA	CAG	GTT	TTT	TTA	CTA	CCG	AGT	C	-5'					
								Asn														
Asp457Asn							3'- CCT	TTA	CAG	GTT	TTT	CTA	TTA	CCG	AGT	CAT	TG	-5'				
								Asn														
Asn462His													3'- CCG	AGT	CAT	TGT	GTA	TGT	AAT	CTT	T -5'	
																	His					
Asn462Asp													3'- CCG	AGT	CAT	TGT	CTA	TGT	AAT	CTT	T -5'	
																	Asp					
Asn462Lys													3'- CCG	AGT	CAT	TGT	TTT	TGT	AAT	CTT	T -5'	
																	Lys					
Asn462Tyr													3'- CCG	AGT	CAT	TGT	ATA	TGT	AAT	CTT	T -5'	
																	Tyr					

FIGURE 1 The oligonucleotide primers and corresponding peptide sequences of variants of PQQGDH-Bs.

TABLE I Substrate specificity of PQQGDH-Bs

	Wild type	Asp448Asn	Asn452Thr	Asp456Asn	Asp457Asn	Asn462His
Glucose	100(%)	100(%)	100(%)	100(%)	100(%)	100(%)
3-O-methyl-glucose	81	72	59	78	80	53
Allose	47	39	33	43	46	32
Galactose	11	14	5	16	11	2
Maltose	61	36	30	41	32	25
Lactose	61	48	31	59	43	31

Each enzyme activity was measured at 20 mM substrate concentration. The values were the relative activity compared with the activity toward glucose as the substrate.

Analysis of Substrate Specificity

Using crude enzyme preparations, wild-type and mutant PQQGDH-B activities towards each substrate were obtained at 20 mM and compared with the activity with 20 mM glucose. More detailed kinetic parameters were then determined using a purified enzyme. Enzyme samples were incubated in 10 mM MOPS-NaOH, pH 7.0, containing 1 mM CaCl_2 and 1 μM PQQ to form a holo enzyme. Enzyme activity was measured as above, except that the concentration of each substrate was varied.

Thermal Stability and EDTA Tolerance

The thermal stability was determined using purified Asn452Thr and wild-type enzyme samples. The time course for thermal inactivation at 55°C was obtained by incubation of each enzyme sample at 0.05 $\mu\text{g ml}^{-1}$ in 10 mM MOPS-NaOH (pH 7.0) buffer. After different incubation, aliquots of the sample were withdrawn, incubated at 4°C for 2 min, and the residual activity was determined at 25°C.

EDTA tolerance of Asn452Thr was determined by incubating purified enzyme in 10 mM MOPS-NaOH (pH 7.0) containing 5 mM EDTA and samples were taken periodically, with their residual activity being expressed.

3D Model Prediction

Three-dimensional structure prediction of mutant enzyme was performed using the Molecular Operating Environment (MOE) (Chemical Computing Group Inc., Quebec, Canada), based on the structural data base of the wild-type enzyme (PDB code; 1QBI, Oubrie *et al.*, 1999a).

RESULTS

Site-directed Mutagenesis Studies on Amino Acid Residues at Loop 6BC Region

Table I summarizes the substrate specificity profiles using crude enzyme preparation of Asp448Asn, Asn452Thr, Asp456Asn, Asp457Asn and Asn462His the mutant PQQGDHs constructed in this study. All the mutants showed different substrate specificity profiles from the wild-type enzyme. The most remarkable differences were observed in the relative activities with maltose and lactose compared with the activity with glucose as the substrate. The relative activities of the wild-type enzyme with maltose and lactose were about 60% compared with the activity with glucose. However, the mutants showed relative activities between 30 and 50% for both substrates compared with the activity with glucose. These results showed that the introduction of a mutation

TABLE II Substrate specificity of Asn452 variants and Asn462 variants

	Wild type	Asn452Thr	Asn452Asp	Asn452His	Asn452Lys	Asn452Ile
Glucose	100(%)	100(%)	100(%)	100(%)	100(%)	100(%)
3-O-methyl-glucose	81	59	92	80	77	92
Allose	47	42	49	42	56	42
Galactose	11	5	19	11	5	8
Maltose	61	30	50	39	42	28
Lactose	61	42	56	39	55	36

	Wild type	Asn462His	Asn462Asp	Asn462Lys	Asn462Tyr
Glucose	100(%)	100(%)	100(%)	100(%)	100(%)
3-O-methyl-glucose	81	53	80	92	92
Allose	47	32	37	55	49
Galactose	11	2	6	13	13
Maltose	61	25	41	47	52
Lactose	61	31	52	60	66

Each enzyme activity was measured at 20 mM substrate concentration. The values were the relative activity compared with the activity toward glucose as the substrate.

TABLE III Kinetic parameters of PQQGDH-Bs for various substrates

	Wild type				Asn452Thr				Asn462His			
	<i>K_m</i> (mM)	<i>V_{max}</i> (U/mg)	<i>k_{cat}</i> (s ⁻¹)	<i>k_{cat}/K_m</i> (s ⁻¹ mM ⁻¹)	<i>K_m</i> (mM)	<i>V_{max}</i> (U/mg)	<i>k_{cat}</i> (s ⁻¹)	<i>k_{cat}/K_m</i> (s ⁻¹ mM ⁻¹)	<i>K_m</i> (mM)	<i>V_{max}</i> (U/mg)	<i>k_{cat}</i> (s ⁻¹)	<i>k_{cat}/K_m</i> (s ⁻¹ mM ⁻¹)
Glucose	25.0	4610	3860	154	12.5	2139	1791	143	12.3	1672	1399	114
3-O-methyl-glucose	28.7	3596	3011	105	27.6	1497	1253	45	28.8	1271	1064	37
Allose	35.5	2997	2509	71	38.7	1134	949	25	32.5	1237	1035	32
Galactose	5.3	277	232	44	3.7	86	72	23	2.7	83	69	26
Maltose	26.0	2305	1930	74	46.5	1197	1002	22	16.0	702	588	37
Lactose	18.9	1982	1659	88	33.6	1240	1038	31	18.0	686	574	32

into the 6BC loop region can lead to an alteration of the substrate specificity profile of GDH-B. Because the Asn452Thr and Asn462His mutants showed the lowest relative activities for maltose and lactose, we constructed a series of variants by modifying the residue a position 452 to Asp, His, Lys and Ile and the residue at position 462 to Asp, Lys and Tyr. The substrate specificity profile of each mutant was then compared to that of the wild-type.

The Asn452Asp and wild-type enzyme were found to have similar substrate specificities. However, the other 4 Asn452 variants, Asn452His, Asn452Lys, Asn452Ile and Asn452Thr, showed much narrower substrate specificity profiles. The relative activities of these mutants with maltose and lactose were about 30–40% compared with the activity with glucose. On the other hand, among the Asn462 variant only Asn462His showed a narrower substrate specificity profile. All other Asn462 variants showed similar profiles to the wild-type enzyme (Table II). Because Asn452Thr and Asn462His showed the least narrowed substrate specificity profiles among these mutants, further detailed analysis were carried out using these two mutants.

Characterization of Asn452Thr

Table III shows the kinetic parameters obtained for Asn452Thr and Asn462His. Both mutants showed smaller *K_m* values for glucose (Asn452Thr: 12.5 mM, Asn462His: 12.3 mM) than wild-type enzyme (25 mM). The *V_{max}* values for glucose of these two mutants were also smaller than that of the wild-type enzyme. As a result, the catalytic efficiency (*k_{cat}/K_m*) values with glucose for Asn452Thr and Asn462His were similar to that of the wild-type enzyme. However, the substrate specificity profiles of these mutants, evaluated based on the relative catalytic efficiencies with 3-*O*-*m*-glucose, allose, maltose, lactose and galactose, were significantly different (Fig. 2). As was observed in the experiment using crude enzyme samples and 20 mM substrate concentrations the relative activities of mutant enzymes with maltose and lactose were lower than that of the wild-type enzyme. Relative to the activity with glucose, the catalytic efficiencies of Asn452Thr with maltose and lactose were 15 and 22%, respectively, while catalytic efficiencies of Asn462His were 33 and 30%, respectively. The altered substrate specificity profile based on the comparison of the catalytic efficiencies of Asn452Thr was found to be mainly due to increase in *K_m* values for the substrates other than glucose (maltose: 26–46.5 mM, lactose: 18.9–33.6 mM). However, the *K_m* values with maltose and lactose of Asn462His were similar to those of the wild-type enzyme. The altered substrate specificity profile of Asn462His was instead found to be due to decreased *V_{max}* values

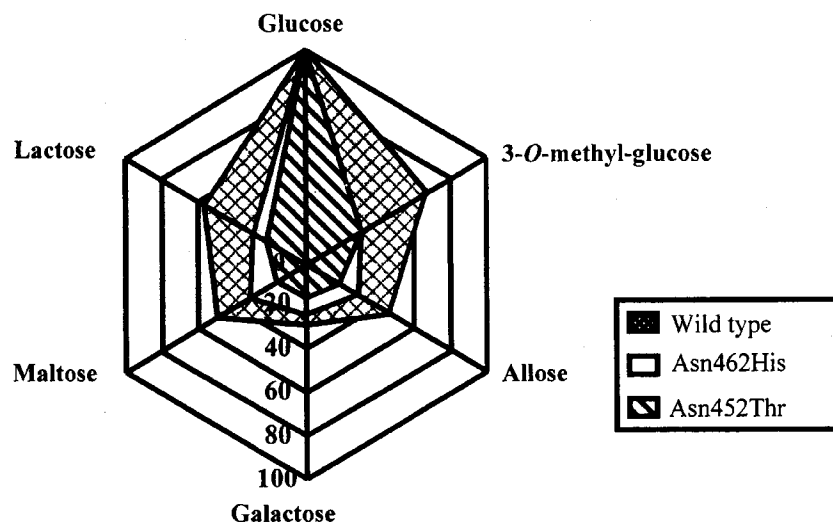


FIGURE 2 Substrate specificity of PQQGDH-Bs. Substrate specificity of each enzyme is shown by comparing catalytic efficiency (kcat/Km) toward each substrate. The catalytic efficiency toward glucose is taken as 100% for each enzyme.

with maltose and lactose and a decreased K_m value for glucose.

The Asn452 residue is located in the center of loop 6BC, where no amino acid residue interacts with glucose. Since the side chain of Asn452 as well as Asn452Thr faced the active site cavity, the side chain of Thr may cause the steric hindrance toward the approach of disaccharides, such as lactose and maltose, to the active site cavity.

We then investigated the thermal stability and EDTA tolerance of Asn452Thr. For the binding of co-factor, PQQ, binding of bivalent metal ions to the active site is essential in all PQQ harboring enzymes. Therefore, the evaluation of EDTA tolerance in

the PQQ enzymes represents the stability of the co-factor binding. Both the thermal stability and EDTA tolerance of Asn452Thr were at similar level as the wild-type enzyme (Figs. 3 and 4).

These results indicated that Asn452Thr was an engineered enzyme with narrower substrate specificity while retaining a similar catalytic efficiency, thermal stability and EDTA tolerance as the wild-type enzyme.

Glucose Measurement Using Asn452Thr

Because Asn452Thr showed narrower substrate specificity than the wild-type enzyme, and particularly

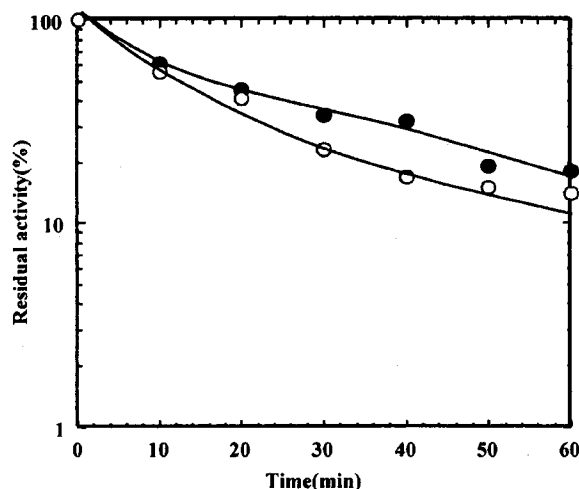


FIGURE 3 Thermal stability of wild-type and Asn452Thr PQQGDH-Bs at 55°C. (●) wild-type enzyme ($0.05 \mu\text{g}^{-1} \text{ml}^{-1}$); (○) Asn452Thr ($0.05 \mu\text{g}^{-1} \text{ml}^{-1}$). After a period of incubation, an aliquot of the sample was withdrawn, incubated at 4°C for 2 min, and the residual activity determined at 25°C.

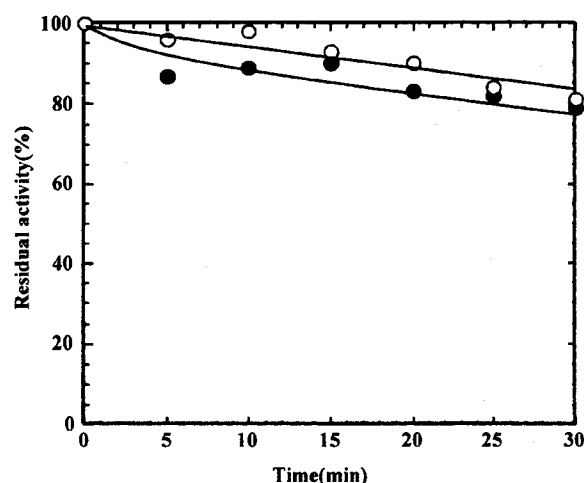


FIGURE 4 EDTA tolerance of wild-type enzyme and Asn452Thr PQQGDH-Bs. In the presence of 5 mM EDTA at 25°C, the time course of residual activity was measured. The initial activities showed in the absence of EDTA were used as a control. (●) wild-type enzyme; (○) Asn452Thr.

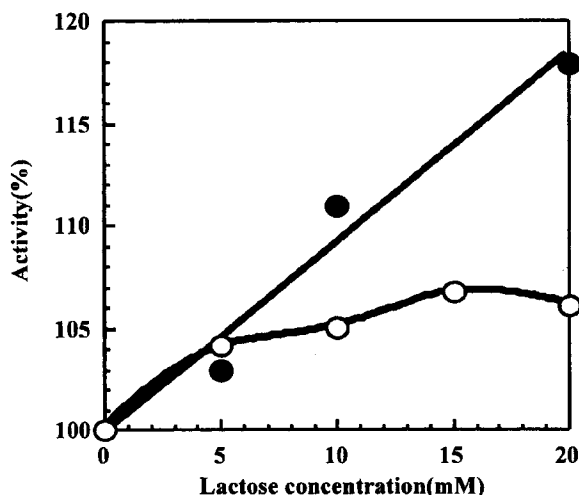


FIGURE 5 The effect of the presence of lactose on glucose measurement using both PQQGDH-Bs, wild-type enzyme and Asn452Thr. Samples were pre-incubated 30 min in the presence of 1 μ M PQQ and 1 mM CaCl_2 before measurement. PMS/DCIP were used as the electron acceptor. The rate of reaction toward 10 mM glucose, in the absence of lactose, was presented as 100%.

showed lower catalytic efficiency toward lactose ($31 \text{ s}^{-1} \text{ mM}^{-1}$) compared with those of wild-type enzyme ($88 \text{ s}^{-1} \text{ mM}^{-1}$) and those of Asn452Thr toward glucose ($143 \text{ s}^{-1} \text{ mM}^{-1}$), we investigated the effect of the presence of lactose on the glucose measurement using both wild-type PQQGDH-B and Asn452Thr (Fig. 5). When using the wild-type enzyme, with the increase of lactose concentration, the observed discoloring reaction of DCIP toward 10 mM glucose increased. No saturation was observed up to 25 mM lactose. In the presence of 10 mM lactose, the effect was greater than 10%. This influence was due to the relatively high catalytic efficiency toward lactose ($88 \text{ s}^{-1} \text{ mM}^{-1}$) compared with that for glucose ($154 \text{ s}^{-1} \text{ mM}^{-1}$). In contrast, when using Asn452Thr to measure glucose, the addition of 25 mM lactose only caused a 5% increase in the apparent rate of the reaction. Furthermore, saturation was reached at around 15 mM lactose concentration. This was due to the lower catalytic efficiency for lactose and higher catalytic efficiency for glucose compared with the wild-type enzyme. Considering that Asn452Thr showed lower $k_{\text{cat}}/K_{\text{m}}$ values toward other sugars like maltose and galactose than those of wild-type enzyme, the engineered enzyme appears to have great potential for measuring glucose as the signal is only minimally affected by the presence of other sugars.

DISCUSSION

Reports on the engineering of PQQGDH-B remain limited. We previously reported on the construction

of PQQGDH-B mutants with improved thermal stability or catalytic efficiency. Ser231Lys was found to have a greater thermal stability while possessing a similar catalytic efficiency and substrate specificity as the wild-type enzyme. This was not surprising since the site where we introduced the amino acid substitution was the opposite site of the cavity where the substrate binding site and catalytic center are located. Glu277Lys was shown to have an increased catalytic efficiency compared to the wild-type enzyme. Unfortunately, the amino acid substitution also resulted in wider substrate specificity than the wild-type enzyme by decreasing K_{m} values and increasing k_{cat} values for all the substrates. Glu277 is located in the 4BC loop, one of the loop regions making up the cavity that interacts with glucose at the active site cleft. None of the Glu277 mutant enzymes showed narrowed substrate specificity.

In this study, we introduced amino acid substitutions within the 6BC loop region, which makes up the active site cleft without directly interacting with the substrate. We expected that the amino acid substitutions in the 6BC loop might form new interactions either with the substrate or with other loop/strand regions. This would hopefully result in an active site cleft with an altered shape that may consequently have altered substrate specificity. Figures 6 and 7 show the predicted 3D structures of the active sites of Asn452Thr and the wild-type enzyme, respectively, in the presence of glucose or lactose. The distance from the 4th hydroxyl of glucose to Thr452 and Asn452 are 7.4 and 6.5 Å, respectively. In both cases, the distances are too great for an interaction to occur. The most significant impact of the amino acid substitution is observed in the interaction between the 6BC loop region with lactose. In the wild-type enzyme, the distance between Asn452 and the 3rd O at the non-reducing end of lactose is 2.4 Å and may form a hydrogen bond. However, the Asn452Thr amino acid substitution resulted in the elimination of interaction between the 6BC loop region and the non-reducing end of lactose. The distance between Thr452 and the 3rd O is 5.2 Å and that with the 2nd O is 4.8 Å. This alteration in the interaction with lactose probably accounts for the decrease in the activity with lactose as well as causing an alteration of loop-loop interactions between the 6BC loop and the 4D5A loop. In the wild-type enzyme, the distance between Asn452 in the 6BC loop and Tyr367 in the 4D5A loop is 2.7 Å, close enough to form a hydrogen bond. However, by substituting Asn452 with Thr, the side chain of the 452nd residue does not face the 4D5A loop and consequently the distance from Tyr367 is increased to 4.0 Å. In Asn452Thr, the distance between Tyr367 and the 4th hydroxyl group of glucose is 3.9 Å, which is closer than the 4.4 Å

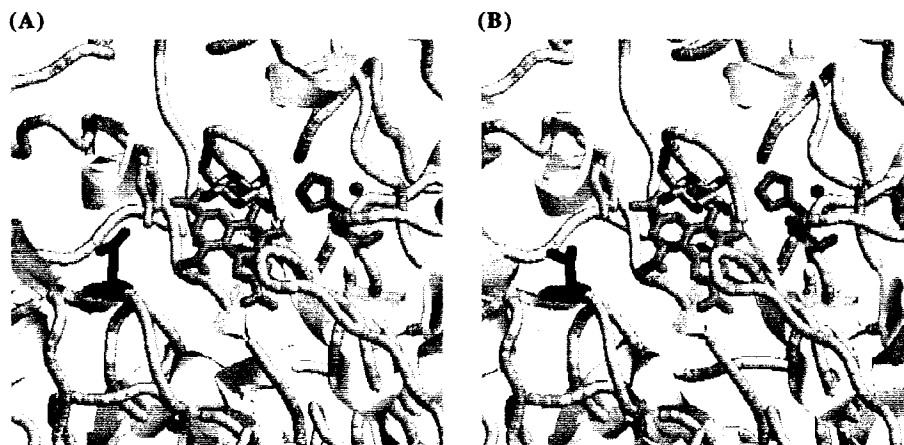


FIGURE 6 The predicted structure of the active sites of wild-type(A) enzyme and Asn452Thr(B) in the presence of glucose. Green residues; His168, Yellow residues; Tyr367, Blue residue; Asn452, Red residue; Thr452, Cyan molecule; PQQ, Violet sphere; Ca ions.

distance in the wild-type enzyme. The side chain position of Tyr367 facing the 4th hydroxyl group of glucose may cause steric hindrance preventing lactose entering the cavity. The distance between the first hydroxyl group and His168, which is the active site residue functioning as the general base, is 2.6 Å, which is identical to that of the wild-type enzyme. This is consistent with the almost identical catalytic efficiencies with glucose of the wild-type enzyme ($155 \text{ s}^{-1} \text{ mM}^{-1}$) and Asn452Thr ($143 \text{ s}^{-1} \text{ mM}^{-1}$). However, the distance between His168 and the first hydroxyl group of lactose is larger in the Asn452Thr mutant (3.9 Å) than in the wild-type (3.2 Å). The increase in the distance between the active site and lactose probably caused the decrease in the catalytic efficiency with this substrate. Because the Asn452Thr mutation had no significant effect on the interaction with glucose, the substrate specificity toward glucose increased versus

lactose. The mutant enzyme shows equivalent catalytic efficiency compared with the wild-type enzyme. The catalytic efficiency of PQQGDH-B toward glucose (wild-type; $155 \text{ s}^{-1} \text{ mM}^{-1}$, Asn452Thr; $143 \text{ s}^{-1} \text{ mM}^{-1}$) is far superior to those of GOD (300 U mg^{-1} , $K_m = 30 \text{ mM}$, $\text{MW} = 186 \text{ kDa}$ dimeric enzyme with one active site in each monomer, $k_{\text{cat}} = 46 \text{ s}^{-1}$, $k_{\text{cat}}/K_m = 1.5 \text{ s}^{-1} \text{ mM}^{-1}$). Although the substrate specificity of GOD is still superior to mutant PQQGDH-B (e.g. commercial available GOD does not catalyze the oxidation of disaccharides such as lactose and maltose), the far superior catalytic efficiency of mutant PQQGDH-B makes it valuable as a glucose sensor component.

In conclusion, this is the first study that has achieved a narrowing of the substrate specificity of PQQGDH-B. Further combinations of mutations, such as with the Ser231 to Lys substitution, may result in the formation of PQQGDH with superior

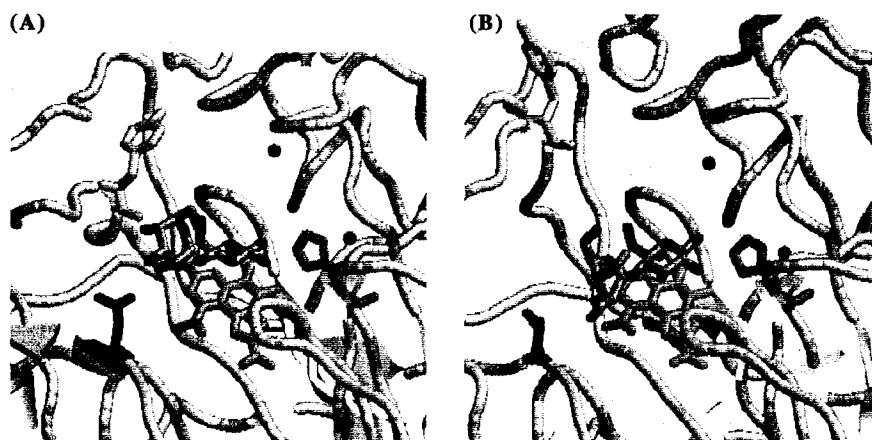


FIGURE 7 The predicted structure of the active sites of wild-type(A) enzyme and Asn452Thr(B) in the presence of lactose. Green residues; His168, Yellow residues; Tyr367, Blue residue; Asn452, Red residue; Thr452, Cyan molecule; PQQ, Violet sphere; Ca ions.

characteristics, which may have great potential for their utilization in enzyme based glucose sensor construction.

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